Video Article

Application of Granger Causality Analysis of the Directed Functional Connection in Alzheimer's Disease and Mild Cognitive Impairment

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URL: <https://www.jove.com/video/56015>

DOI: [doi:10.3791/56015](https://doi.org/10.3791/56015)

Keywords: Medicine, Issue 126, Alzheimer's disease, Granger causality analysis, resting-state functional brain imaging, directed functional connectivity, default mode network, posterior cingulate cortex

Date Published: 8/7/2017

Citation: Wang, M., Liao, Z., Mao, D., Zhang, Q., Li, Y., Yu, E., Ding, Z. Application of Granger Causality Analysis of the Directed Functional Connection in Alzheimer's Disease and Mild Cognitive Impairment. *J. Vis. Exp.* (126), e56015, doi:10.3791/56015 (2017).

Abstract

Impaired functional connectivity in the Default Mode Network (DMN) may be involved in the progression of Alzheimer's Disease (AD). The Posterior Cingulate Cortex (PCC) is a potential imaging marker for monitoring the progression of AD. Previous studies did not focus on the functional connectivity between the PCC and nodes in regions outside the DMN, but our study is an effort to explore these overlooked functional connections. For collecting data, we used functional Magnetic Resonance Imaging (fMRI) and Granger Causality Analysis (GCA). fMRI provides a non-invasive method for studying the dynamic interactions between the different brain regions. GCA is a statistical hypothesis test for determining whether one-time series is useful in forecasting another. In simple terms, it is judged by comparing the "Known all the information on the last moment, the distribution of the probability of X at this time" and the "Known all the information on the last moment except Y, the distribution of the probability of X at this time", to determine whether there is a causal relationship between Y and X. This definition is based on the complete information source and stationary chronological sequence. The main step of this analysis is to use X and Y to establish the regression equation and draw a causal relationship by a hypothetical test. Since GCA can measure causal effects, we used it to investigate the anisotropy of the functional connectivity and explore the hub function of the PCC. Here, we screened 116 participants for MRI scanning, and after preprocessing the data obtained from neuroimaging, we used GCA to derive the causal relationship of each node. Finally, we concluded that the directed connection is significantly different between the Mild Cognitive Impairment (MCI) and AD groups, both from the PCC to the whole brain and from the whole brain to the PCC.

Video Link

The video component of this article can be found at <https://www.jove.com/video/56015/>

Introduction

AD is a degenerative disease of the central nervous system that can be diagnosed using histopathology, electrophysiology, and neuroimaging¹. The memory-related DMN is a vital system of the interacting brain regions associated with AD, and its abnormal function is characteristic of AD^{2,3}. The PCC is an important region of the traditional default network in the resting state and plays pivotal roles in episodic memory, spatial attention, self-evaluation, and other cognitive functions^{4,5,6,7}. In addition, it might be an imaging marker for monitoring AD progression. Using GCA, Liao *et al.* found that the PCC is a region of multiple cytoarchitectonics with multiple connections and plays an important role in functional brain structure⁸. Zhong *et al.* reported that the PCC was a convergence center that received interactions from most of the other regions within the DMN³. Furthermore, Miao *et al.* demonstrated that in the DMN hub regions, the PCC has the greatest causal effect relationship with other nodes⁹. Together, all this evidence indicates that the directed connection of the PCC is valuable in AD research and the PCC needs to be further studied in-depth as a vital region of the DMN.

The previous studies were confined to the connectivity between the PCC and other regions within the DMN; however, the changes in directed functional connectivity between the PCC and brain regions outside the DMN, as well as their influence on AD have not yet been explored¹⁰. Our study further investigated this unexplored functional connectivity in normal healthy controls, patients with MCI, and patients with AD. By observing the directed connectivity between the PCC and whole brain regions, we aimed to elucidate the functional changes in the brain related to AD progression, and thereby establish a novel objective basis for assessing the severity of the disease.

Functional connectivity refers to an interregional interaction that can be represented by synchronous Low Frequency Fluctuations (LFFs) in the cerebral Blood Oxygen Level Dependent (BOLD) fMRI signal. Therefore, in order to observe the functional connectivity between the PCC and other brain regions, we analyzed the functional connectivity between the PCC and the whole brain network by fMRI using GCA, with the PCC as the Region of Interest (ROI). This technique directly derives the fundamental relationship of each node using data obtained from neuroimaging¹¹.

Recently, GCA has been applied to electroencephalogram (EEG) and fMRI studies to reveal the causal effects among brain regions¹². All these studies indicated that the GCA technique might be optimal for detecting the causal relationship of each node in the brain.

Protocol

This study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital. Every enrolled subject signed a written informed consent.

1. Sample Classification and Screening

1. Diagnose and divide 116 patients into AD and MCI groups.
NOTE: Use the 2011 National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria and the Mini-Mental State Examination (MMSE) criteria for identification and classification of MCI, which is described in detail in Yu *et al.*¹⁰
2. Select participants in the healthy control group.
NOTE: The age, gender, and education level of the control group were matched with patients in the MCI and AD groups.
3. Assess all subjects by MMSE¹⁰.
4. Exclude the subjects who did not fulfill the inclusion criteria. For all subjects, the exclusion criteria are mentioned in Yu *et al.*¹⁰
5. Use MRI to scan subjects; exclude subjects with unusable data due to head movements.
NOTE: Ultimately, we screened 26 patients with MCI, 32 patients with AD, and 58 controls.

2. Acquisition of Neuroimaging

1. Remove metal and magnetic objects before entering the 3.0 T MRI Laboratory.
2. Install an MRI receiving coil at the magnetic resonance scanning bed. The receiving coil is an 8 channel circularly polarized brain phased-array coil.
3. Instruct the participant to lie on the bed, and to remain supine with the head advanced and the long axis of the body along the long axis of the bed. Place the participant's head on the bracket of the coil, and make sure that the orbitomeatal line is perpendicular to bed.
4. Tell the participant to place the upper limbs to the sides of the body, keep eyes closed, not think of anything in particular, and move as little as possible during the scan. Place foam pads on the head in the bilateral temporal area to prevent head movement and provide headphones to reduce scanner noise for the participant.
NOTE: Dimensions of the foam pad are: long diameter = 13 cm, short diameter = 10 cm, the thickest thickness = 7 cm, the thinnest thickness = 3 cm, average thickness = 5 cm.
5. Adjust the position of the head through the positioning light so that the sagittal positioning cursor is in the midline of the face, and the axis positioning cursor is parallel to the lateral canthus. Then move the bed to keep the axis positioning cursor on the eyebrows or 2 cm below it.
6. Move the head to the center of the magnet. Perform an fMRI brain scan, including gradient Echo-Planar Imaging (EPI-BOLD) and 3D-T1-MPRAGE imaging.
NOTE: Use the following parameters:
EPI-BOLD: TR/TE = 2,000/30 ms, layer thickness/layer pitch = 3.2/0.8 mm, 31 slices, matrix = 64 x 64, FOV = 220 x 220 mm, voxel size = 3 x 3 x 4 mm, flip angle = 90 °, scanning time of 484 s, and a total of 240 scanning images.
3D-T1-MPRAGE imaging: TR/TE = 8.5/3.2 ms, flip angle = 15 °, field of view = 250 x 250 mm, matrix = 256 x 256, slice number = 176, slice thickness/gap = 1/0 mm, scanning time of 353 s, and a total of 192 scanning images.
7. Keep the patient safe when they are leaving the bed at the end of the scan.

3. Data Preprocessing

NOTE: Analyze the raw data for resting-state brain functions by using the Resting-State fMRI (rs-fMRI) Data Analysis Toolkit plus (RESTplus).

1. Open RESTplus through MATLAB and left click on **Pipeline**.
2. Import the relevant files into RESTplus. Select the work directory and then the starting EPI and T1 directories.
3. Convert DICOM files to NIFTI. Check off the **DICOM to NIFTI** box in preprocessing and check off the **EPI DICOM to NIFTU** and the **T1 DICOM to NIFTI** parameters.
4. Remove the first 10 time points by checking off the **Remove first n time points** and setting the n parameter as **10**.
5. Set the slice timing according to rs-fMRI parameters. Check off the **Slice timing** box. Set the slice number according to the rs-fMRI parameters of the study. Enter the slice order.
NOTE: The acquisition of data of each layer in the brain scan is not at the same time point, and thus, it needs to be calibrated to the same time point.
6. Correct the time and head motion. Check off **Realign**.
NOTE: The exclusion criterion for excessive head motion was >2.0 mm translation or >2.0 ° rotation in any direction. In the RESTplus this is a default parameter (left click on the option of 'Realign').
7. Perform spatial normalization by using T1 image unified segmentation and all heads standardized to the same space. Check off **Normalize** and leave the default parameters at the bottom. Select the **Normalize by using T1 image unified segmentation** and **European** parameters.
NOTE: Resample the rs-fMRI images with voxels of 3 x 3 x 3 mm, and other parameters in the RESTplus are default, just left click on the option of 'Normalize by using T1 image unified segmentation'.
8. Perform spatial smoothing using an isotropic Gaussian kernel with a full-width at half maximum (FWHM) of 6 mm. Check off **Smooth**.
9. Remove the linear trend by checking off **Detrend**.

10. Regress out signals from nuisance regressors (WM, CSF, Global) to increase signal-to-noise ratio. Check off **Nuisance covariates regression** and the following parameters: **6 head motion parameters, global mean signal, white matter signal, and cerebrospinal fluid signal**.
NOTE: During this step, set the 'Polynomial trend' as 1 as default, and choose the '6 head motion parameters', the 'Nuisance regressors (WM, CSF, Global)' and the 'add mean back' as default.
11. Use band-pass filtering to retain signals between 0.01 - 0.08 Hz. Remove high-frequency physiological noise, and low-frequency drift. Check off **Filter**.

4. Directed Connectivity Analysis

NOTE: Perform GCA combined with the BOLD signals for each voxel in the whole brain after extracting the average BOLD signal intensity in the seed area.

1. Perform the voxel-wise GCA by using the REST-GCA in the REST toolbox. In the post-processing box, check off **GCA**.
2. Set the 'order' as 1 as default. Select the parameters in the input.
3. Define ROI and identify seed points of interest in the PCC. Select **Define ROI** and choose the Spherical ROI. Select **Next**. Set the center coordinates and radius of the seed ROI based on the known data and select **OK**.
NOTE: An ROI for the DMN was placed at the PCC (centering at $x = 0$, $y = -53$, $z = 26$ with radius = 6 mm), as in a previous study¹³.
4. Select **Run** and **OK** to run the program.
5. Find folders named ZGCA and GCA after processing of relevant file data. Sort out the files of ZGCA and classify them into four subfolders, xx, xy, yx, yy accordingly.
NOTE: Later, mainly use the xy and yx subfolders. The three sets of file data ('AD' 'MCI' 'NC') are all processed and sorted according to steps 3.1 - 4.5 above.
6. Open RESTplus through MATLAB and left click on **Statistical Analysis**. Left click on **REST Two-Sample T-Test**.
7. Name the output result as T1xy and set the output directory. Left click on **Add Group Images** to open the xy subfolder in the AD Results folder and the xy subfolder in the NC Results folder.
8. In the option of **Mask File**, left click to open the BrainMask_05_61*73*61 subfile in the 'mask' folder.
9. Select **Compute** to run the program.
10. Name the output result as T2xy and set the output directory. Left click on **Add Group Images** to open the xy subfolder in the AD Results folder and the xy subfolder in the MCI Results folder. Repeat steps 4.8 - 4.9.
11. Name the output result as T3xy and set the output directory. Left click on **Add Group Images** to open the xy subfolder in the MCI Results folder and the xy subfolder in the NC Results folder. Repeat steps 4.8 - 4.9.
12. Name the output result as T1yx and set the output directory. Left click on **Add Group Images** to open the yx subfolder in the AD Results folder and the yx subfolder in the NC Results folder. Repeat steps 4.8 - 4.9.
13. Name the output result as T2yx and set the output directory. Left click on **Add Group Images** to open the yx subfolder in the AD Results folder and the yx subfolder in the MCI Results folder. Repeat steps 4.8 - 4.9.
14. Name the output result as T3yx and set the output directory. Left click on **Add Group Images** to open the yx subfolder in the MCI Results folder and the yx subfolder in the NC Results folder. Repeat steps 4.8 - 4.9.
15. Finally, obtain the six result files by following steps 4.6 - 4.14 and left click on **viewer** of RESTplus to view the result. Import the template named ch2 in **Underlay**.
16. Find the six result files in the output directory and fill in the **Overlay** one by one. Obtain the final result graph, and the six result files that correspond to the six graphs.
17. Use Statistical Product and Service Solutions (SPSS) to process the data obtained from the previous step.
 1. Present Continuous variables as means and Standard Deviations (SD).
 2. Present categorical variables as numbers and percentages, then use the chi-square test.
 NOTE: All p -values of <0.05 were considered statistically significant.

Representative Results

Demographic information

Table 1 presents the characteristics of the subjects. All the subjects had an education level of junior school or above. Age, gender, and education level were similar between the three groups ($P > 0.05$), while the MMSE scores were significantly different ($p < 0.01$).

Directed brain functional connectivity

The active nodes in the whole brain were first identified. Using the GCA technology, the directed functional connectivity from the PCC to the whole brain and from the whole brain to the PCC in the AD, MCI, and control groups were determined (**Figure 1, Figure 2; Tables 2, 3**).

The directed connectivity from the whole brain to the PCC was enhanced in the AD group as compared to the normal control group, and primarily focused in the bilateral cerebellar region outside the DMN. The directed connectivity from the PCC to the whole brain was significantly reduced in the AD group as compared to the controls, with the main regions, such as the right precuneus and left middle frontal gyrus, belonging to the DMN.

The directed functional connectivity from the PCC to the whole brain in the bilateral precuneus and postcentral gyrus regions showed a significantly enhanced causal effect in the MCI group as compared to the controls. The directed functional connectivity from the whole brain to the PCC was not significantly enhanced in the MCI group compared to the controls; however, it was significantly reduced in the regions outside the DMN including the caudate nucleus, putamen, and parietal lobule.

The directed connections were significantly different between the MCI and AD groups, both from the PCC to the whole brain and from the whole brain to the PCC. Furthermore, these differences were predominantly observed in the left hemisphere (**Tables 2, 3**), probably indicating asymmetry. The abnormally directed connectivity in the left hemisphere was primarily located in the DMN. With regard to the differences in the directed connections between the MCI and AD groups, more left hemisphere regions (with a significant causal effect) were involved in the connectivity from the PCC to the whole brain than the regions involved in the connectivity from the whole brain to the PCC.

Items	MCI	AD	NC	χ^2 or F	P -value
Enrolled cases (n)	26	32	58	-	-
Gender (male/female)	10/16	13/19	23/35	0.028 ^a	0.986
Age (years)	71.64±6.03	71.62±5.85	69.87±6.04	1.25 ^b	0.290
Years of education (years)	9.82±2.03	9.63±1.96	9.76±1.87	0.08 ^b	0.926
MMSE score (points)	24.32±1.74	18.58±1.86	29.0±1.58	392.31 ^b	<0.001

MMSE: Mini-Mental State Examination, NC: Normal Control. a: χ^2 value; b: F value.

Table 1: Characteristics of the MCI, AD, and Control Groups.

The characteristics of the subjects: age, gender, and education level were similar between the 3 groups, while the MMSE scores were significantly different.

Brain region	MNI space	Brodmann	Peak T	Cluster size (voxels)
	(X Y Z)	area	score	
AD vs. NC				
Left middle frontal gyrus	-30,45,0	10/11	-7.86	204
Right inferior frontal gyrus	57,39,6	46	6.65	93
Right precuneus	3,-54,45	7/31	-7.3	223
MCI vs. NC				
Right cerebellum	30,-63,-48	NaN	-8.52	92
Right superior frontal gyrus	30,63,9	10/46	-6.8	86
Left precuneus/postcentral	-15,-36,78	3/7	6.38	82
Right precuneus/ postcentral	27,-27,72	4/3	7.03	81
AD vs. MCI				
Left cerebellum	-30,-39,-42	NaN	8.55	136
Left middle temporal	-33,-15,-27	20/21	8.09	216
Left caudate/lentiform nucleus	-9,15,6	NaN	-7.02	110
Right precuneus/calcarine	15,-75,15	18/31	-8.03	127
Left precuneus	-12,-57,27	31	7.32	127
Left middle frontal gyrus	-30,12,63	6/8	8.23	86
Left precentral	-21,-36,72	3/7	-8.18	87

Table 2: PCC to the Whole Brain.

(a) The directed connection from the PCC to the whole brain: the directed connections were significantly different between the MCI and AD groups, from the PCC to the whole brain. (b) These differences were predominantly observed in the left hemisphere, possibly indicating asymmetry.

Brain region	MNI space	Brodmann area	Peak Z	Cluster size (voxels)
	(X Y Z)		score	
AD vs. NC				
Right cerebellum	51,-75,-27	NaN	7.03	88
Left cerebellum	-24,-87,-30	NaN	10	138
Left middle frontal gyrus	-30,45,0	NaN	8.3	261
Left angular convolution	-39,-63,36	39/40	-6.69	98
MCI vs. NC				
Left putamen	-24,6,-6	NaN	-7.69	77
Left caudate nucleus	12,9,6	NaN	-6.42	122
Left superior parietal lobule	-33,-63,51	7	-9.31	154
AD vs. MCI				
Left putamen	-27,9,-9	NaN	4.2	85
Right putamen	21,12,-6	NaN	4.93	100
Left precuneus	-18,-63,27	31	-4.12	41
Left superior parietal lobule	-21,-60,48	7	4.7	42

Table 3: Whole Brain to the PCC.

(a) The directed connection from PCC to the whole brain: the directed connections were significantly different between the MCI and AD groups, from the whole brain to the PCC. (b) These differences were predominantly observed in the left hemisphere, possibly indicating asymmetry.

Figure 1: PCC to the Whole Brain.

The directed connection from the PCC to the whole brain. The active nodes in the whole brain were first identified, and the directed functional connectivity from the PCC to the whole brain in AD, MCI, and control groups was determined. [Please click here to view a larger version of this figure.](#)

Figure 2: Whole Brain to the PCC.

The directed connection from whole brain to the PCC. The active nodes in the whole brain were first identified, and the directed functional connectivity from the whole brain to the PCC in AD, MCI, and control groups was determined. [Please click here to view a larger version of this figure.](#)

Discussion

This report presents a process for comparing the directed functional connectivity from the PCC to the whole brain and from the whole brain to the PCC between AD, MCI and control groups. Moreover, a key step in this process is the classification and screening of sample before the experiment. Thus, the classification and screening criteria are crucial because the accuracy of the results can be affected if they are erroneous. As listed in the protocol, we used 2011 NINCDS-ADRDA diagnostic criteria and MMSE, and the criteria for identification and classification of MCI; our screening criteria are also referred to in the above protocol. We excluded those patients who were not suitable for the trial, and then, accurately classified the remaining patients, which is fundamental for subsequent experiments. The critical GCA analysis derives the directed functional connectivity from the PCC to the whole brain and from the whole brain to the PCC between the AD, MCI, and control groups using the data obtained from neuroimaging. We presented the details of the GCA analysis in this protocol. This technology found significant differences between the groups in directed connectivity, and explained well the correlation between the functional changes and AD progression.

In our data analysis of this study, differences occurred between individuals (not including age, gender, and education levels, since these are similar between the 3 groups, and hence, it is difficult to make an objective evaluation of the conclusions). To resolve this issue, we analyze the data of the groups, rather than the individual data. The variables in each group are represented as the mean and SD, and the continuous variables as numbers and percentages, using the chi-square test. Through this quantitative assessment, we can objectively evaluate the directed connectivity between the PCC and the entire brain region and elucidate the functional changes associated with the progression of AD in the brain.

Although linear correlation and Independent Component Analysis (ICA) have been widely used to study the functional connectivity, these results have no directivity. GCA can be utilized not only to measure the causal effects of fMRI time series but also to show the dynamics and direction of the BOLD signal obtained from rs-fMRI^{14,15}.

We analyzed the directed connectivity between the PCC and the whole brain network using GCA with PCC as the ROI and found differences in directed connectivity between the AD, MCI, and control groups. Thus, we concluded that PCC, as an important hub of the DMN brain region, has a significant impact on AD progression. PCC may not only show abnormalities in the receiving information but also show abnormalities in transmitting information. In addition, this study shows that the transmission of information in all brain regions with abnormal connections is directional, except for individual nodes (left middle frontal gyrus and left precuneus), since the abnormalities in these brain regions are unilateral. Another interesting finding of this study is that these connection anomalies seem to occur mainly in the left hemisphere. This may be because

the dominant hemisphere (left hemisphere) is more susceptible to injury than the right hemisphere, leading to early metabolic decline and atrophy¹⁶.

Nevertheless, there are some limitations in our study. For GCA technology, when the sampling rate reaches 2 s, different hemodynamic delays are difficult to obtain accurately¹⁷, and the slow dynamics of the BOLD signal at 2 s can cause some rapid causal relationship loss¹⁸. As a result, this limitation can lead to the deviation of the experimental data. Since the test sample size was not sufficiently large enough, additional samples are essential to verify the results.

Currently, some studies have used multivariate GCA techniques to describe the causal relationships between multiple brain regions. In theory, multivariate GCA is an improved technique that can reveal the complexity of directional connectivity in the brain; however, it has more technical challenges than bivariate GCA as hemodynamic delay varies with the region¹². Our future goal is to address the challenges of multivariate GCA and apply it to the research to better demonstrate the complex directional connectivity of the brain.

Disclosures

The authors declare that they do not have any competing financial interests.

Acknowledgements

The authors thank Gongjun Ji for computer software support. This research was partially supported by the National Natural Science Foundation of China (no. 81201156, 81271517); the Zhejiang Provincial Natural Science Foundation of China (no. LY16H180007, LY13H180016, 2013C33G1360236), and the Science Foundation from the Health Commission of Zhejiang Province (no. 2013RCA001, 201522257).

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